

Recent advances in physiological and practical aspects of ectomycorrhizal effects on tree development

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ABSTRACT

After a general review of mycorrhizal symbioses, this paper deals with the beneficial aspects on tree growth of ectomycorrhizae and their possible utilization in nursery practices. Several causes are usually given for explaining the beneficial role of ectomycorrhizae, one being the increase of soil volume explored by the mycorrhizae; the mycorrhizal structure also improves nutrient uptake, water absorption and protection against pathogens. For these reasons artificial inoculations of forest nurseries may afford considerable potential benefits for tree growth. However, before ectomycorrhizal inoculation becomes a routine nursery practice, several problems need further study, particularly the selection of fungi, and the production of large quantities of viable mycelial inoculum.

KEY WORDS: ectomycorrhizae, forest nursery, inoculation, inoculum production, nitrogen metabolism, phosphorus metabolism.

RÉSUMÉ

Après avoir considéré quelques aspects généraux concernant la symbiose ectomycorhizienne, cet article s'intéresse aux aspects favorables des ectomycorhizes sur la croissance des arbres et leur utilisation en pépinière. Ces effets favorables des mycorhizes s'expliquent généralement par un accroissement du volume de sol exploré par les racines mais aussi par l'amélioration de l'alimentation minérale, de l'absorption d'eau et de la protection contre les pathogènes. Il est alors évident que l'inoculation artificielle des pépinières forestières ne peut qu'avoir des effets bénéfiques sur la croissance des arbres. Cependant, pour devenir une pratique courante en pépinière, l'inoculation ectomycorhizienne doit être éprouvée notamment en ce qui concerne la sélection de l'espèce fongique et la production de grande quantité d'inoculum mycélien.

MOTS CLÉS : ectomycorhizes, pépinière forestière, inoculation, production d'inoculum, métabolisme azoté, métabolisme phosphoré.

INTRODUCTION

During the last century there has been a great increase of interest in mycorrhiza, a symbiotic association between plant and fungus. Authors postulate that the original evolution of terrestrial plants from an aquatic habit was possible only through a mutualistic relationship between green algae and fungi (TRAPPE, 1977).

Though it was the german botanist FRANK who, in 1885, gave the name mycorrhiza to associations between host roots and fungi, there had been previous observations of them. FRIES towards the middle of the 18th century noticed the presence of fungal hyphae on the root surface of *Monotropa*, and TULASNE at the same period described a fungal layer formed by truffle mycelia around tree roots. These observations confirmed that made by THEOPHRASTE some 2000 years ago who had remarked the curious arrangement of fungal species around wood species roots (quoted by BOULLARD, 1968).

FRANK (1887) designated ectotrophic and endotrophic mycorrhizae as the principal types:

— Ectotrophic mycorrhizae, now termed ectomycorrhizae, consist of infected short roots covered with a mantle of hyphae. From this mantle the hyphae extend externally in all directions in the soil, as well as intercellularly in the primary cortex of the root to form the so-called Hartig net (fig. 1).

— Endotrophic mycorrhizae, now named endomycorrhizae, cannot be identified from exterior signs since the fungus penetrates the root cells without forming an external mantle (fig. 1). MELIN (1927) defined ectendotrophic mycorrhizae (now ectendomycorrhizae) as an intermediate form with both a typical Hartig net and intracellular hyphae. This paper deals only with ectomycorrhizal associations.

In temperate regions, most woody plants are dependent on ectomycorrhizal fungi. The important forest species include both gymnosperms, e. g. fir, hemlock, larch, pine, spruce, and many angiosperms, e. g. beech, birch, oak, poplar.

Fungi associated with these hosts are mostly basidiomycetes such as *Amanita*, *Lactarius*, *Pisolithus*, *Rhizopogon*, although some ascomycetes form mycorrhizae e. g. *Cenococcum*, *Elaphomyces*, *Tuber*. A single tree species can have numerous species of fungi capable of forming ectomycorrhizae on its roots. Thus TRAPPE estimated that some 2000 species of fungi are apparently host-specific (e. g. *Suillus grevillei* on larch), whereas others have broad host ranges (e. g. *Cenococcum geophilum*) (MOUSAIN, 1976; MARX, 1977; TRAPPE, 1977).

Since the discovery of mycorrhizae, much has been learnt of their structure, distribution, physiology and ecology (ROUQUEROL, 1967; MOSSE *et al.*, 1981; HARLEY & SMITH, 1983; KOTTKE & OBERWINKLER, 1986; MARTIN *et al.*, 1987), allowing investigators not only to ask fundamental questions about physiological functioning and ecological significance of ectomycorrhizal symbioses, but also to make use of them in nursery practices and forest production.

The objective of this paper is to discuss the practical aspects of ectomycorrhizal inoculation in nurseries, having previously described the benefits of establishing such an association for host growth and metabolism.

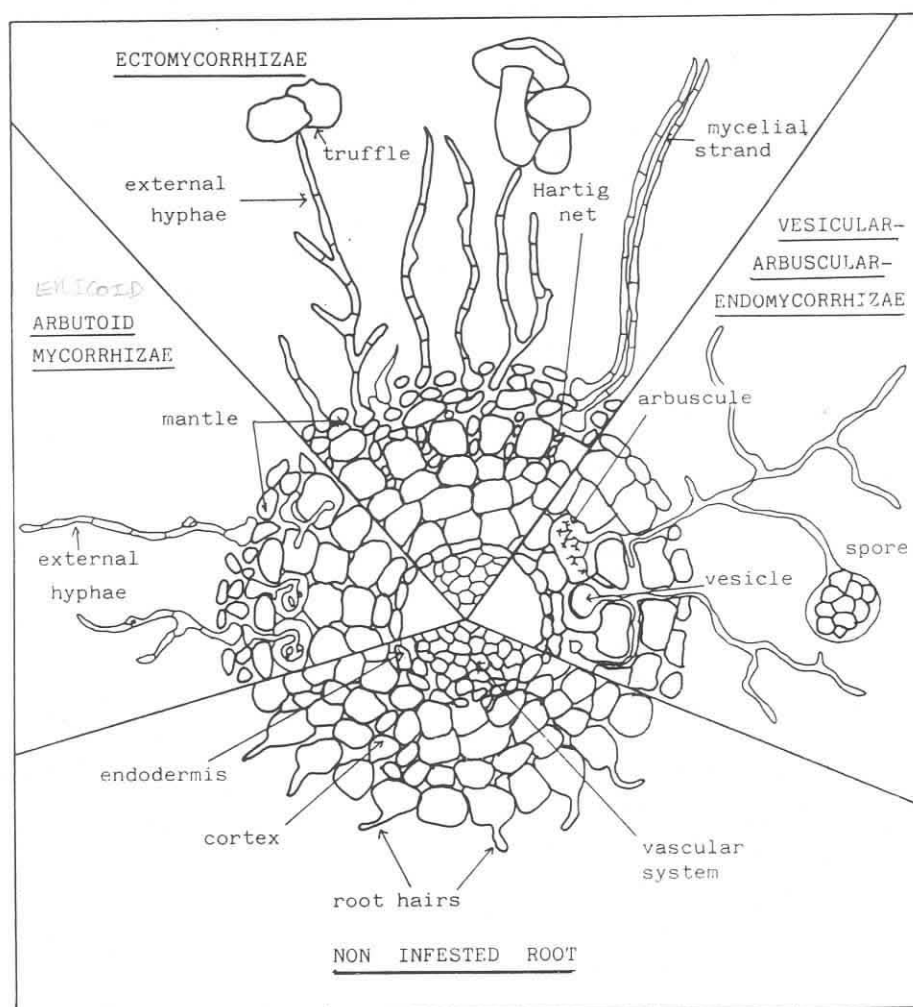


FIG. 1. — Schematic representation of mycorrhizal types
(Redrawn from LE TACON, 1985).

I. FAVOURABLE EFFECTS OF MYCORRHIZAE ON PLANT GROWTH

(A) Nutritional effects

Experiments performed in the first half of this century (e.g. HATCH, 1937) indicated that ectomycorrhizal seedlings were not only larger in size, but also contained greater quantities of the major nutrients such as nitrogen, potassium, phosphorus per unit weight than non-mycorrhizal controls (Table I). Researchers then attempted to explain this beneficial effect of mycorrhizae on host growth and metabolism (e.g. ROUQUEROL, 1967).

TABLE I. — *Changes in growth and chemical characteristics of Pinus strobus seedlings, due to inoculation of mycorrhizal fungi to the soil (from HATCH, 1937).*

Treatments	Nitrogen			Phosphorus		Potassium	
	Total dry wt. (mg)	% of dry wt. (mg)	Total per plant (mg)	% of dry wt. (mg)	Total per plant (mg)	% of dry wt. (mg)	Total per plant (mg)
Non infected controls	302.7	0.84	2.69	0.074	0.236	0.425	1.38
Inoculated plants	404.6	1.24	5.00	0.196	0.789	0.744	3.02

1. Increase of absorbing surface

To explain the importance of mycorrhizae in their increase nutrient uptake, HATCH (1937) put forward an hypothesis that the absorbing surface of the root system was extended following infection. The fungi associated with the roots produce external hyphae which allow the host to draw on nutrients far away from roots within a radius of 20 cm or more.

The use of isotopic tracers later confirmed this hypothesis. KRAMER and WILBURG (1949) demonstrated by using radioactive phosphorus that mycorrhizal short roots of *Pinus* accumulate larger quantities of phosphorus than do uninfected roots. Similar results were obtained with excised beech tips by HARLEY and MAC CREADY (1952). MELIN and NILSSON (1958) also conclusively demonstrated that mycorrhizal mycelia are capable of transferring nutrients from a distant source to the roots of pine seedlings, by using radioactive phosphorus (^{32}P) and the stable nitrogen isotope (^{15}N), as well as metallic ions.

2. Phosphate nutrition

The central role of the fungal symbiont in the greater efficiency of phosphorus uptake is now well established (HARLEY & SMITH, 1983). Using ^{32}P , HARLEY and MAC CREADY (1952), showed a rapid incorporation of this isotope in the nucleotide and sugar phosphate pools. Phosphorus plays a major role in transfer of genetic information being part of nucleic acids (DNA and RNA), in energetic processes being part of ATP and also in regulation processes being part of AMPc.

Phosphate assimilation by mycorrhizal roots has been divided into three major steps (HARLEY & SMITH, 1983; GIANINAZZI-PEARSON & GIANINAZZI, 1985):

— *Mobilization and absorption:* Ectomycorrhizal trees can utilize some sources of phosphate unavailable to non-mycorrhizal ones. Organic or mineral insoluble forms of phosphorus seem to be mobilized by ectomycorrhizae.

WILLIAMSON and ALEXANDER (1975) reported that there was 2-8 times more phosphatase activity on the surface of mycorrhizal beech roots than on non-mycorrhizal roots. Such activity might hydrolyse organic phosphorus. THEODOROU (1968) shown that several mycorrhizal fungi are able to dissolve Ca-phytate, due to phytase activity rather than to pH changes in the medium.

Pinus sylvestris seedlings grown with ^{32}P labelled Fe-phosphate, apatite and humus, absorbed more labelled P when mycorrhizal with *Suillus luteus* than when non-mycorrhizal or mycorrhizal with *Amanita muscaria* (RITTER & LYR, 1965). Similar results were obtained by MEJSTRIK & KRAUSE in 1973. *Pinus radiata*

mycorrhizal with *Suillus luteus* took up more ^{32}P from labelled apatite and humus than when it was non-mycorrhizal or mycorrhizal with *Cenococcum geophilum*.

Absorption is an active and metabolically dependent process, being temperature and azide sensitive dependent and requiring oxygen (HARLEY *et al.*, 1958).

— *Accumulation*: Polyphosphate compounds are a major reserve in ectomycorrhizal fungi and represent up to 30% of total phosphate according to MARTIN *et al.* (1985). Many workers have also detected P-accumulation in mycorrhizae as polyphosphate granules in the fungal sheath (CHILVERS & HARLEY, 1980; LAPEYRIE *et al.*, 1984).

Polyphosphate is compartmentalized in the fungal vacuole and forms meta-chromatic inclusions that can be visualized by transmission electronic microscopy (STRULLU *et al.*, 1983).

The ability of the fungal sheath in ectomycorrhiza to accumulate large amounts of phosphate as polyphosphate and rapidly mobilize them when required makes them a potentially important storage organ for the host plant.

— *Transfer to the host tissues*: In vesicular-arbuscular mycorrhizae, phosphorus transfer to the host is presumed to be coupled with carbohydrate transfer to the fungus (DEXHEIMER *et al.*, 1985). If comparable mechanisms have not yet been demonstrated in ectomycorrhizae, recent investigations have clearly shown that the Hartig net is organized in a way that bidirectional transfer of ions and molecules is promoted (KOTTKE & OBERWINKLER, 1987). This organization consists in lack of septation of the hyphae and their intimate juxtaposition, resulting in a coenocytic structure, functionally equivalent to the transfer cells in vascular plants.

These subjects have been reviewed by HARLEY and SMITH (1983) but more research is needed concerning processes occurring at the soil-fungus and fungus-host interfaces.

3. Nitrogen nutrition

Nitrogen is one of the most important nutrient limiting growth in forest systems and is required by trees for normal growth and development (KRAMER & KOZLOWSKI, 1979). It also appears to be the most important nutrient in increasing forest productivity.

Most investigations of the nitrogen nutrition of ectomycorrhizal plants have examined the assimilation of mineral nitrogen including nitrate and ammonium forms (CARRODUS, 1966, 1967; PLASSARD *et al.*, 1985; MARTIN *et al.*, 1986) or simple amino-N compounds (MELIN & NILSSON, 1953; STRIBLEY & READ, 1980; SANGWANIT & BLEDSOE, 1985). Although, it is clearly shown that mycorrhizae stimulate nitrate and ammonium uptake by woody plants (HARLEY & SMITH, 1983; RYGIEWICZ *et al.*, 1984a and b), the translocation within the fungus and between the fungus and the host is still not explained.

Ectomycorrhizal fungi can be involved in different stages of nitrogen metabolism as described by FRANCE and REID (1983) (fig. 2). Absorbed ammonium is assimilated into organic compounds via two pathways (PLASSARD *et al.*, 1985). An NADP-dependent glutamate dehydrogenase (GDH) which catalyses the amination of oxoglutarate giving glutamate; the GS/GOGAT pathway with the sequential enzymes: glutamine synthetase (GS) which catalyses the amination of glutamic acid

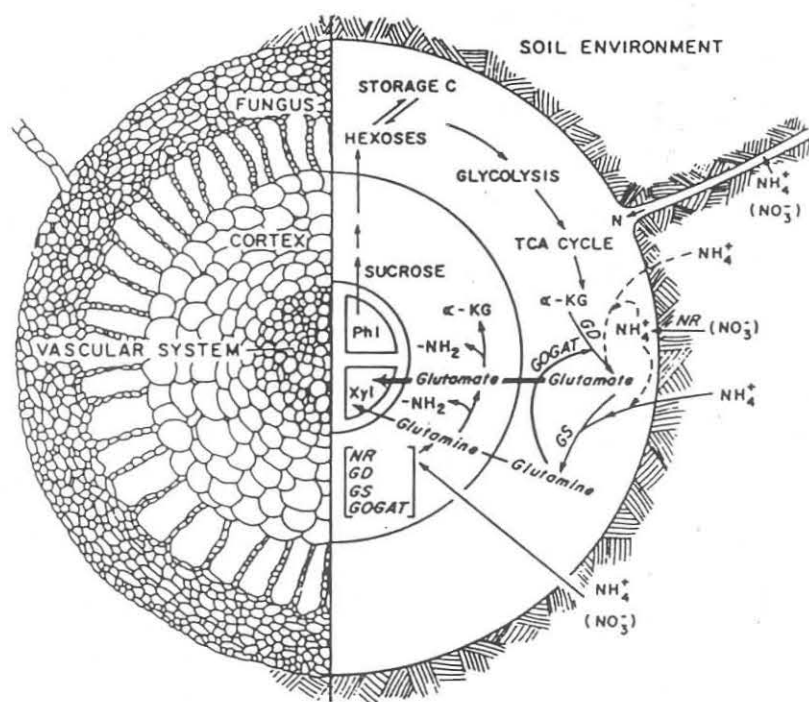


FIG. 2. — Conceptual model of carbon and nitrogen interactions in the ectomycorrhiza. Relative importance of flows are indicated by line widths; solid lines are predominant flows as compared with broken lines. Lesser importance of nitrate absorption indicated by parentheses. NR, nitrate reductase system; GD, glutamate dehydrogenase; GS, glutamine synthetase; GOGAT, glutamate synthase; α-KG, α-ketoglutarate; TCA cycle, tricarboxylic acid cycle (FRANCE & REID, 1983).

giving glutamine and glutamate synthase (GOGAT) which catalyses the formation of glutamate from glutamine.

The GDH pathway predominates in fungi (PATEMAN & KINGHORN, 1975; MARZLUF, 1987) and has been shown to occur in the ectomycorrhizal fungus *Cenococcum geophilum* (MARTIN *et al.*, 1983). The GS/GOGAT pathway is the major route of NH₄ assimilation in higher plant roots (MIFLIN *et al.*, 1981; OAKS & HIREL, 1985). The nitrogen assimilation pathways have not yet been fully elucidated in ectomycorrhizae although both the GS/GOGAT pathway and the GDH pathway of nitrogen assimilation are potentially operative since the association contains both fungal and higher plant cells.

Recent work using ¹⁵N nuclear magnetic resonance spectroscopy is consistent with a minor role for fungal GDH in ectomycorrhizal ammonia assimilation (MARTIN *et al.*, 1986). The results of these authors suggest either the action of fungal GS and host GOGAT with translocation of glutamine and glutamate between the cells of the two partners, or the absorption of ammonium by the

fungal hyphae and its translocation to the host cells where it is incorporated into the amino acid by the usual GS/GOGAT host pathway.

Even if most investigations of the nitrogen nutrition of ectomycorrhizal plants take into consideration the simple form of nitrogen, recent results propose that colonization might provide access to organic nitrogen compounds which constitute the major source of this nutrient in organic forest soils. ABUZINADAH and READ (1986), found that in the absence of infection, birch, black spruce and pine were completely unable to use protein as a nitrogen source, whereas when mycorrhizal all species readily used protein nitrogen producing vigorous shoots (fig. 3).

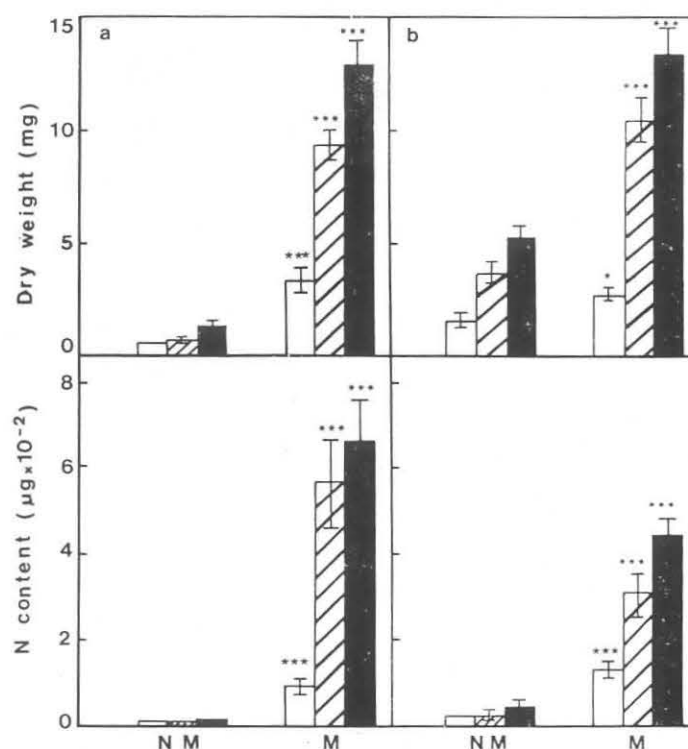


FIG. 3. — Mean dry weights (above) and nitrogen contents (below) of roots (□), shoots (▨) and whole plants (■) of *Betula pendula* after growth for 60 days in the mycorrhizal (M) and non-mycorrhizal (NM) condition with the protein BSA as sole exogenous nitrogen source. Asterisks indicate the significance of differences between M and NM treatment means: ***P < 0.001; *P < 0.05. Vertical bars indicate standard errors. (b) as in (a) but for *Picea mariana* (Redrawn from ABUZINADAH & READ, 1986).

The ability to use proteins as a source of nitrogen requires the intervention of proteases, enzymes able to depolymerase those compounds. Such a protease activity has already been reported in mycelial extracts and culture filtrates of a few mycorrhizal fungi (RAMSTEDT & SODERHALL, 1983; BOTTON *et al.*, 1985).

As already demonstrated for *Neurospora crassa* (ABBOTT & MARZLUF, 1984), secretion of proteases can be induced when supplying the nutrient medium with commercial protein (casein, gelatin or bovine serum albumine) or more efficiently with proteins extracted from soil in the case of ectomycorrhizal fungi such as *Lactarius subdulcis* and *Amanita rubescens* (EL-BADAoui & BOTTON, unpublished results).

4. Water uptake

Some workers noticed that ectomycorrhizal infection can lead not only to enhancement of plant nutrient content but also to additional uptake of water. Thus BOYD *et al.* (1985) demonstrated the capacity of fungal mycelium to supply water in sufficient quantities to sustain transpiration and photosynthesis. Such ectomycorrhizal plants are according to THEODOROU and BOWEN (1970) more resistant to drought stress.

(B) NON NUTRITIONAL MYCORRHIZAL EFFECTS

1. Protective effect against pathogens

Some ectomycorrhizal fungi constitute a potential of biological control, against pathogens (MARX, 1972; TRAPPE, 1977). *Laccaria laccata* has been reported to be very effective in protecting roots of Douglas fir and spruce seedlings against *Fusarium oxysporum* (SINCLAIR *et al.*, 1982). However, mechanisms involved in the protective role of ectomycorrhizal fungi are not well understood, though several possibilities have been suggested by MARX (1972), e.g. physical protection by the mantle, competition with the pathogen for sugars from the roots, stimulation of an antagonistic microflora associated with the mantle, production of antimicrobial metabolites or antibiotics. In recent papers, this last hypothesis seems to be the best explanation since an increased production of inhibitory substances such as terpenes and phenolics by the host has been shown (SAMPANGI & PERRIN, 1985).

MARX (1973) has examined the potential of mycorrhizal fungi to inhibit pathogens. He found that several strains of *Leucopaxillus cerealis* produced substances antagonistic to *Phytophthora cinnamomi* on all media, and identified them as nitrile (MARX, 1975).

2. Stimulating effect on metabolite production

Investigators have emphasized that mycorrhizal fungi also supply vitamins and growth stimulating substances such as auxins, gibberellins and cytokinins. SHEMAKHANOVA (1962) demonstrated the production of vitamins such as biotin, thiamine, panthotenic acid by numerous ectomycorrhizal fungi. MILLER (1967) isolated cytokinins (e.g. zeatin) from culture filtrate of *Rhizopogon roseolus*. Since the discovery of ectomycorrhizae hyperauxiny by MAC DOUGAL and DUFRENOY (1944), indole compound release, by ectomycorrhizal fungi in pure culture, has been studied by different workers (SLANKIS, 1958; MOSER, 1959; ULRICH, 1960; GOGALA, 1971; GAY *et al.*, 1985; ROUILLON *et al.*, 1986; HO & TRAPPE, 1987).

Most of the fungi studied synthesized indole compounds, particularly IAA, but the amount of auxins produced, their composition, and the time necessary for their production in detectable amounts differed among different species.

The correlation between the ability of a fungus to release these compounds and his morphogenetic effect on host roots is not yet well established but should undoubtedly clarify the role of these metabolites in the establishment of the symbiotic relationship.

II. PRACTICAL USE OF MYCORRHIZAE

1. Introduction

Artificial regeneration using direct sowing or planting of young plants from nurseries plays a major role in reforestation practices. Mycorrhizal symbioses can enhance tree growth but foresters and nursery men will have to promote their synthesis and subsequent development. Inoculating host species with ectomycorrhizal fungi may be done in the nursery (TRAPPE, 1977), thus improving tree survival and growth when the seedlings are planted on forest sites (MARX, 1977).

Even if vigorous, non-mycorrhizal seedlings are produced on a fertilized soil, the use of frequent applications of concentrated inorganic fertilizers leads to a progressive independence of forest plants from mycorrhizal fungi. As LE TACON (1982) pointed out, the higher is the fertilizer level, the fewer are the symbionts that remain and the more fertilizers are needed. The effects of soil fertility on root infection by ectomycorrhizal fungi were described in the early literature. HATCH (1937) affirmed that a favourable development of mycorrhizal roots required a soil low in nutrients such as nitrogen, phosphorus, potassium or calcium. BJÖRKMAN (1942) indicated that low nutrient levels in soil promoted ectomycorrhizal development.

More recently MARX *et al.* (1977) demonstrated the effect of soil fertility on internal concentrations of soluble carbohydrates in *Pinus* roots and their effects on the susceptibility of these roots to colonization by ectomycorrhizal fungi (Table II).

TABLE II. — Growth measurements, inorganic chemical analyses of needles and root carbohydrate analyses of non-mycorrhizal Loblolly pine seedlings after 10 weeks at 2 levels of soil fertility, and their susceptibility to *Pisolithus tinctorius* (from MARX *et al.*, 1977).

Measurement and soil treatment	Height (cm)	Total dry wt. (mg)	Needle analyses (mg/g tissues)		Root carbohydrate analyses (μmol/g root)		% mycorrhizae
			Nitrogen	Phosphorus	Sucrose	Fructose	
Low fertility level	7.6	151	10.3	2.7	1.56	0.75	19.0
High fertility level	11.2	519	17.3	3.2	1.49	0.38	3.5

These results indicate that high levels of nitrogen and phosphorus in soil decrease the sucrose content of short roots of Loblolly pine which in turn decreases their susceptibility to ectomycorrhizal development by *Pisolithus tinctorius*.

Soil fumigation, a presowing practice used routinely in some conventional tree nurseries, usually eliminates or drastically reduces most of the microbial population in the top 20 or 30 cm of soil (MEXAL, 1980; LE TACON & GARBAYE, 1986). Pathogens are eliminated but many desirable organisms including mycorrhizal fungi

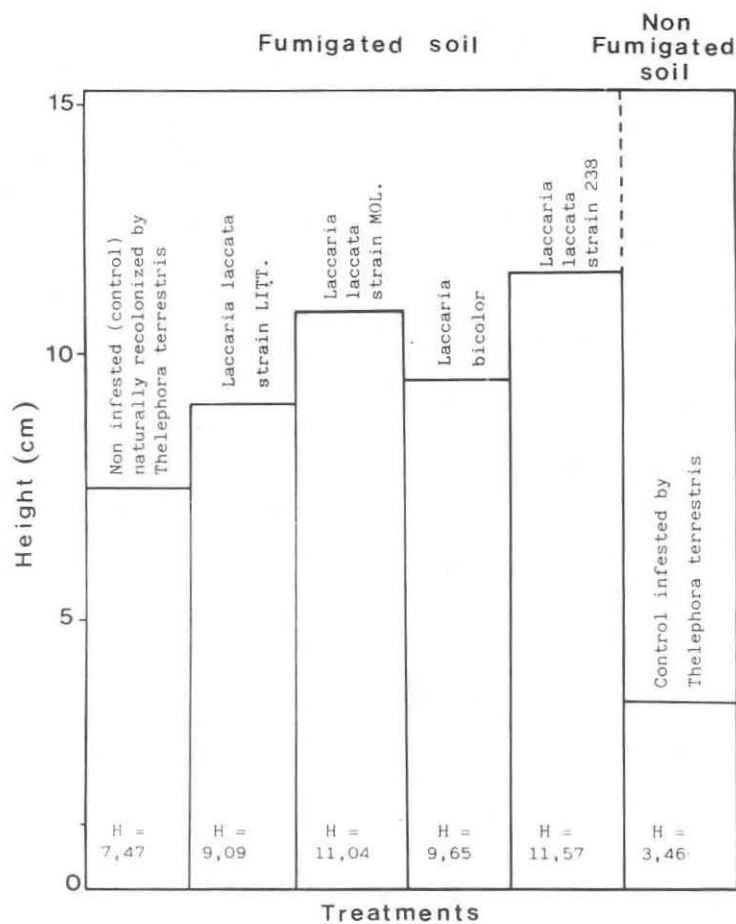


FIG. 4. — Effect of soil fumigation and ectomycorrhizal inoculation on growth of Douglas-fir, six months old (Redrawn from LE TACON & GARBAYE, 1986).

may also be eliminated. The slow and erratic recolonization may not always be satisfactory since it may be by inefficient mycorrhizal species.

Most data published (RICHARD, 1973; MARX & BRYAN, 1975; LE TACON, 1982; LE TACON & GARBAYE, 1986) show the necessity of inoculation of fumigated nursery soil, not only to sustain the limiting recolonization processes but also to establish selected mycorrhizal fungi. Figure 4 illustrates results obtained in a nursery with Douglas-Fir. Soil fumigation without inoculation allows a significant gain in growth (7,47 cm) compared to non-fumigated soil (3,46 cm) after natural recolonization by *Thelephora terrestris*. Fumigation combined with inoculation of selected fungi (e. g. *Laccaria laccata* strain 238) further improves growth (LE TACON & GARBAYE, 1986).

2. Types of inoculum

Current methods for production of mycorrhizal seedlings have been reviewed by TRAPPE (1977). The use of soil or humus as source of inoculum was practised in the mid 19th century for truffle culture and has been a simple and successful method of mycorrhizal inoculation of nurseries. However this technique could result in the introduction of pathogens as well as the desired fungi (TRAPPE, 1977; LE TACON, 1982).

Transplantation of mycorrhizal seedlings or freshly excised mycorrhizal root systems into nursery beds has been used successfully, but phytosanitary controls still remain difficult (MARX & BRYAN, 1975).

Spores can be used for inoculation since basidiospores can be easily collected from mature sporocarps of some ectomycorrhizal gasteromycetes. This technique is perhaps the most practical inoculation technique (THEODOROU & BOWEN, 1970; MARX & BRYAN, 1975). Unfortunately not all ectomycorrhizal fungal species produce sporocarps and those that do, do not always produce fruit-bodies in quantities sufficient to make field harvest practical.

Inoculation with fungal mycelia was developed in the early 1950's by MOSER in Austria and appeared to be very successful (MARX *et al.*, 1978; MITCHELL *et al.*, 1984; HUNG & MOLINA, 1986; LE TACON & BOUCHARD, 1986). Mycelium is grown in a vermiculite and peat mixture saturated with a nutrient medium (MARX & BRYAN, 1975). With optimal aseptic conditions, this method offers good success for fungi that grow well in culture in the absence of host roots.

TABLE III. — Growth of eight-month old loblolly pine seedlings in fumigated soil with and without *Pisolithus tinctorius* under pine straw soil (from MARX & BRYAN, 1975).

Measurements and soil treatments	Height (cm)	Stem diameter (mm)	Foliar stem dry wt. (g)	Root dry wt. (g)	% mycorrhizae
Infested with basidiospores of <i>P. tinctorius</i>	30.9	6.9	5.6	1.5	57.5
Infested with vegetative mycelium of <i>P. tinctorius</i>	37.5	8.4	8.9	2.6	90.4
Non-infested (controls)	24.9	5.6	3.8	1.3	44.5

Advantages and disadvantages of these two last techniques have been discussed in detail in literature (MARX & BRYAN, 1975; TRAPPE, 1977; MEXAL, 1980). Results obtained by MARX and BRYAN showed that mycelium appears to be an excellent form of inoculum for *Pisolithus tinctorius*, rapidly colonizing the roots and precluding colonization of roots by other ectomycorrhizal fungi including *Thelephora terrestris*. This contrasts with results obtained with basidiospore inoculum of *Pisolithus tinctorius* which did not dominate root systems to the same degree as the vegetative mycelium (Table III).

3. Production of inoculum

Practical and common use of mycorrhizae in nurseries on a large scale could only be regarded as feasible if sufficient quantities of viable inoculum are available.

Two methods for producing large quantities of inoculum are currently in use:

(1) Inoculum production on solid medium

This technique consists of growing the fungus in peat-vermiculite moistened with nutrient media. When used in laboratories this does not entail particular difficulties. The largest attempts to produce commercial inoculum have been made in U.S.A. by MARX and coworkers. However they often failed during fermentation processes in large tanks when cultures have become contaminated.

A technique currently used for commercial mushroom inoculum could be promising for ectomycorrhizal fungi. It consists of growing the fungi in a peat-vermiculite mixture moistened with diluted brewers malt. The growth medium is contained in an autoclavable plastic bag that has a porous membrane to allow gaseous exchange. Tests have shown the successful performance of this technique for limited practical use (HUNG & MOLINA, 1986; LE TACON *et al.*, 1987).

(2) Mycelium cultivated in fermentor and entrapped in polymeric gel

Inoculum of ectomycorrhizal fungi can also be prepared by cultivating the fungus in a fermentor and entrapping it in a polymeric gel as proposed by DOMMERGUES *et al.* (1979) for bacteria and JUNG (1980) for different microorganisms. LE TACON *et al.* (1983) have tested *Hebeloma cylindrosporum* entrapped in several types of polymers with spruce and Douglas fir in fumigated and non-fumigated nursery soil (Table IV). Their results show greater efficiency of an

TABLE IV. — Influence of the type of inoculum on the percentage of survival seedlings and growth of *Picea excelsa* in fumigated soil, six months after sowing, inoculated with *Hebeloma cylindrosporum* (LE TACON *et al.*, 1983). NI, non inoculated; A, alginate gel; B, alginate gel + silice I; C, xanthane + caroube gel + silice I; D, peat and moss (non washed).

Measurements	Type of inoculum					ppds (5%)
	NI	A	B	C	D	
Sowing number by 1/2 m	18.5	22.2	17.5	10.7	15.5	10.5
Height (mm)	41.1	55.6	46.3	48.1	45.5	8.1

alginate entrapping gel inoculum on seedling growth compared with the usual vermiculite-peat inoculum. This method of producing mycelium of an ectomycorrhizal fungus in a fermentor is not yet utilized in a large scale but it could possibly be used to produce large quantities of inoculum for commercial use.

4. Selection of fungi

The most important step in any nursery inoculation program is the selection of the inoculant fungus (TRAPPE, 1977; LE TACON & BOUCHARD, 1986). Comparisons of effects of different fungi on survival and growth of seedlings in this context are needed, but encounter difficulties since it is not always easy to isolate the mycorrhizal fungus for using the mycelium inoculum technique. Furthermore many ectomycorrhizal fungi grow so slowly that large scale production of inoculum is not yet feasible, whilst others cannot be grown in pure culture by existing methods. Media for growing mycorrhizal fungi still need much improvement (TRAPPE, 1977; LE TACON, 1982). By using basidiospores as inoculum, the identity of the mycorrhizal

fungi is assured but some species (e. g. *C. geophilum*) being imperfect fungi do not fruit.

Most results reported in the literature (KIFFER, 1974; TRAPPE, 1977; HOLDEN *et al.*, 1983; MITCHELL *et al.*, 1984) show differential growth of various host species

TABLE V. — Effects of different mycorrhizal fungal inocula on mean dry weight and top/root (T/R) ratio of seedlings of three conifers grown in tube containers for 6 months (TRAPPE, 1977).

Fungal inoculum	<i>Pinus ponderosa</i>		<i>Tsuga heterophylla</i>		<i>Pseudotsuga menziesii</i>	
	wt. (g)	T/R	wt. (g)	T/R	wt. (g)	T/R
No inoculum	1.44	1.03	0.74	2.89	1.80	1.61
<i>Hebeloma crustuliniforme</i>	1.05	1.05	0.97	2.69	1.75	1.58
<i>Laccaria laccata</i>	1.25	1.14	0.91	2.81	1.88	1.70
<i>Pisolithus tinctorius</i>	1.25	1.02	1.16	2.18	1.71	1.43
<i>Thelephora terrestris</i>	1.14	1.02	1.03	2.49	1.48	1.27

associated with different mycorrhizal fungi (Table V). Moreover, strains of the same species differ in their effect upon growth of seedlings (HACKSKAYLO & BRUCHET, 1972; LE TACON & GARBAYE, 1986; LE TACON & BOUCHARD, 1986).

MARX (1981) found that of 20 isolates of *Pisolithus tinctorius* from different tree hosts around the world, one was far superior to the others for mycorrhizal synthesis on Southern pine, and some did not produce mycorrhizae with the pines at all.

From these results it is clear that some mycorrhizal fungi are more effective than others with respect to stimulation of host plant growth. The ability of a fungal strain to grow in pure culture and its mycorrhizal efficiency should undoubtedly be tested and considered when selecting mycorrhizal fungi for artificial inoculation.

5. Seedling response in nursery

Numerous tests of mycorrhizal inoculation have been made in nurseries for about fifty years. HATCH (1937), studied the formation of mycorrhizae under specific conditions by doing a series of small-scale nursery experiments. More extensive experiments have allowed investigators to progress in the nursery inoculation techniques, e. g. MOSER's work in Austria on *Pinus cembra* (MOSER, 1956), THEODOROU and BOWEN's work in Australia (THEODOROU & BOWEN, 1970).

For fifteen years, MARX and his collaborators have worked on the ectomycorrhizal fungus *Pisolithus tinctorius*, a symbiont of numerous host species and which is especially effective on *Pinus*. Results show when *Pisolithus tinctorius* is artificially introduced into fumigated soil, it successfully competes with other species of naturally occurring ectomycorrhizal fungi. It can also be a biological tool to improve survival and growth of pines in poor quality reforestation sites, this fungus being easily propagated in the laboratory on solid or liquid media (MARX *et al.*, 1982).

In experiments to determine the effects in a fumigated nursery soil of different fungi on mycorrhizal development and seedling growth of Douglas fir, Norway

spruce, larch and Scots pine, LE TACON and BOUCHARD (1986) found that inoculation with pure cultures of two *Laccaria laccata* isolates and one *Hebeloma crustuliniforme* isolate formed abundant mycorrhizae with the four tree species and greatly stimulated seedling growth during the first two years. The most important practical result of their investigation is the obtaining, after two years growth in a fumigated soil, of transplantable Douglas fir seedlings, inoculated with *Laccaria laccata*. Without such an inoculation, three to four years are usually needed in France to obtain transplantable seedlings.

6. Seedling response in the plantation

Even if effectiveness of a mycorrhizal fungus on host trees in the nursery is of great importance in selecting fungal species, it must not be the only selection criterion. It is therefore of great interest to examine the effect on plant growth of controlled mycorrhizal infection after outplanting. Successful manipulations in nurseries and subsequent impact on tree performance show practical value of some nursery inoculation programs (MARX, 1977; FONTANA *et al.*, 1982; GARBAYE & LE TACON, 1986).

Recent work carried out by STENSTRÖM *et al.* (1985) examined the effect on plant growth in forest sites, of low fertilization and controlled mycorrhizal infection

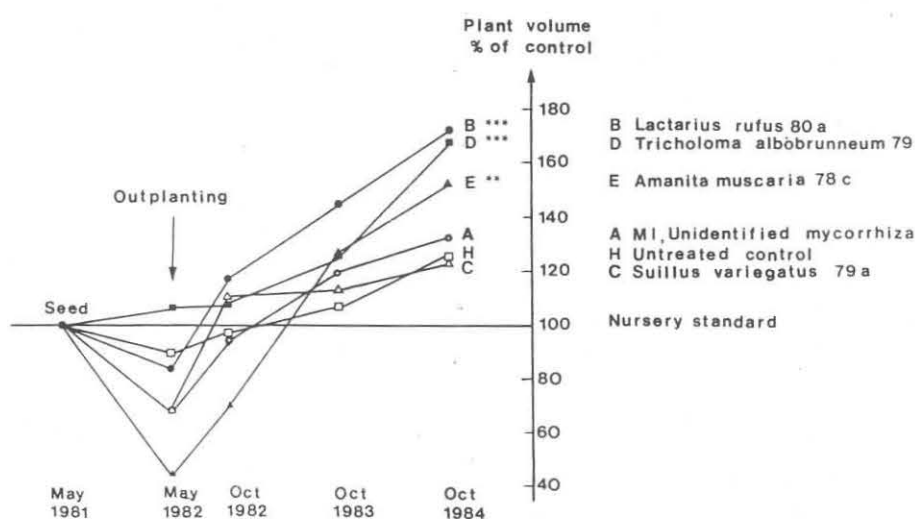


FIG. 5. — Large term effect of initial mycorrhiza and low fertilization on growth of *Pinus sylvestris* plants. Plants were grown for one year, 1981-1982, in the nursery and for more than two years in the field. Growth (diam. \times height) of the low-fertilized plants, inoculated or non-inoculated (control), is compared with those (non-inoculated) having received "nursery standard" fertilization. Differences from the control at the 0.1% level (***) and 1% level (**) are indicated (Redrawn from STENSTRÖM *et al.*, 1985).

in the nursery (fig. 5). Results show that soon after outplanting, the low nutrient plant grew better than the nursery standard. After two and half years in the field, all the mycorrhizal treated plants had out-grown the control by 20 to 75%. Even

the uninfected low nutrient control was 25% bigger than the standard. These results also indicate the greater effectiveness of some species (*Lactarius rufus* or *Tricholoma albobrunneum*) over others (*Suillus variegatus*).

According to the authors, the better growth of mycorrhizal plants could be due to improved utilization of a limited nutrient supply and resistance against pathogens. These data also show that the strains giving the best results after outplanting are not necessarily those giving the best results in nurseries since inoculated and poorly fertilized plants did not grow as well as the uninoculated and normally fertilized standard during the first year in the nursery.

CONCLUSION

Mycorrhizal associations of higher plants are among the most widely known microbial processes in nature. In natural conditions, this symbiosis forms spontaneously and contributes to plant growth.

Nevertheless investigations have proved that natural processes could be improved by manipulating the symbionts to give more beneficial associations, yielding more economic tree growth for foresters.

Towards this, a few efficient strains have already been selected for coniferous host species (*Pisolithus tinctorius* on pine, *Laccaria laccata* on Douglas fir). Improved inoculation techniques in nurseries also have enhanced the possibilities of affecting mycorrhizal development.

However, most of the potential benefits of mycorrhizal strains remain unexplored. For the efficient use of the existing potential not only are more numerous assays and observations in nurseries and plantations needed but also more research on the physiology of mycorrhizae in order to better understand the relations between the plants and their associated fungi. There is a great need for development of methodologies and techniques for study of nutrient uptake and other functions of mycorrhizae which could lead to many useful applications.

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